Protein Biophysics in vivo

2947-Pos Board B102
Protein Folding and Aggregation in the Cell
Simon Ebbinghaus.
Physical Chemistry II, Ruhr University Bochum, Germany.
Cellular protein misfolding and aggregation cause neurodegenerative disorders like Huntington, Parkinson, Alzheimer and prion diseases. The diseases may be predominantly caused by ''gain-of-function'' proteotoxicity, with misfolded proteins prefibrillar and fibrillar aggregates being the toxic species. Protein structure, folding and aggregation kinetics are predominantly investigated in vitro in aqueous solution with powerful biophysical techniques and methods. We study solvent-induced effects on protein aggregation with the focus of understanding the effects of the crowded cellular environment. We use a combination of fluorescence microscopy and temperature jump relaxation (1) to spatio-temporally resolve these events in a single living cell. The same instrument is used to perform the comparative in vitro measurements in dilute buffer, crowded environments and distinct solvents. We present new insights to the in vivo aggregation pathway of intrinsically discorded proteins.
(1) S. Ebbinghaus, A. Dhar, J.D. McDonald and M. Gruebele. Protein folding stability and dynamics imaged in a living cell. Nature Methods, 7:319-323, 2010.

2948-Pos Board B103
Live Cell Fluorescence Correlation Spectroscopy with Real Time Photoactivation Feedback
Matthew D. Weitzman[1], Chandran R. Sabanayagam[2], Kenneth L. van Golen[1].
[1]University of Delaware, Newark, DE, USA, [2]Delaware Biotechnology Institute, Newark, DE, USA.
Fluorescence Correlation Spectroscopy (FCS) is a well established technique, classically used to measure diffusion kinetics in solution. One major hurdle to implementing this technique to *in vivo* applications is the requirement of observing fluorescent molecules in the sub 100nM range. Many cellular proteins are much more abundant than this upper limit, often being expressed at micromolar concentrations. To allow for FCS measurements at these higher physiologically relevant concentrations we have incorporated the use of a photoconvertible fluorophore, mEOS2. This fluorophore initially displays green 488nM spectral characteristics. Under 405nM UV illumination the mEOS2 chromophore undergoes a polypeptide break, shifting it to a red 561nM spectral characteristic. By controlling the intensity and duration of 405nM illumination we have built into our system a feedback mechanism capable of maintaining a defined amount of photo-converted molecules suitable for FCS analysis. We have applied this strategy to study the diffusion characteristics of members of the Rho GTPase subfamily which are endogenously expressed in the micromolar range.

2949-Pos Board B104
Protein Folding across the Cell Cycle
Anna J. Wirth, Martin Gruebele.
University of Illinois at Urbana Champaign, Urbana, IL, USA.
The mammalian cell cycle is the biological pathway through which cells grow and divide. During this physically dramatic process the cell doubles in size, duplicates its genetic material, dissolves its nucleus, and ultimately divides into two genetically identical daughter cells. Due in part to the fundamental role of cell cycle abnormalities to cancer pathogenesis, cell cycle research remains a very active topic in the literature. Yet, there is a comparatively very little work exploring the influence of the cell cycle on protein biophysics. Considering the immense intracellular physical changes the cell undergoes during the cell cycle as well as the established sensitivity of protein folding to the intracellular environment, we aim to determine the *in vivo* protein folding trends as the cell progresses through the stages of the cell cycle. With Fast Relaxation Imaging (FReI), a fluorescence microscopy system for measuring *in vivo* protein folding, we explore the temperature induced folding thermodynamics and kinetics of a FRET-labeled model protein, yeast phosphoglycerate-kinase (PGK), in living U2OS cells arrested in the prometaphase stage of mitosis and the G1 stage of interphase. We see that PGK is more stable in mitotic cells compared to interphase cells, suggesting that the protein folding environment significantly changes as the cell progresses through the cell cycle.

2950-Pos Board B105
Wnt3 Diffuses Freely in the Intercellular Space of Developing Zebrafish Embryos
Guangyu Sun[1], Cathleen Teh[2], Vladimir Korzh[2], Thorsten Wohland[1].
[1]National University of Singapore, Singapore, Singapore, [2]Institute of Molecular and Cell Biology, Singapore, Singapore.
The Wnt family of secreted signaling proteins plays important roles during animal development and disease. Wnt3 is evolutionarily conserved in vertebrates. The zebrafish Wnt3, like that in mice, chick and human is expressed in developing neural tissues. It was shown to activate the canonical Wnt pathway and has been implicated in cell fate determination and stem/progenitor self-renewal. To understand Wnt3 signaling in more detail, it is necessary to study its behavior in cellular compartments as well as in intercellular space. Here, Fluorescence Correlation Spectroscopy (FCS) is applied to investigate *in vivo* Wnt3 secretion and diffusion patterns during zebrafish neural development. We show that the bulk of GFP-tagged Wnt3 is found on the plasma membrane and in the cytosol of Wnt3-producing cells. Wnt3 distribution at the membrane remains relatively constant independent of developmental stages, brain regions and level of expression, indicating that the plasma membrane may act as a first checkpoint for Wnt3 release. The mobility of the membrane-bound Wnt3 increases during development, suggesting faster cargo exchange, which may reflect a dynamic mechanism responsible for Wnt3 export. using spatially resolved FCS we further show that Wnt3 is detected in the fourth ventricle of the hindbrain, forming a gradient from the hindbrain Wnt3 expressing cells to the brain ventricular space. This indicates that Wnt3 freely diffuses from Wnt3-producing cells and may traverse a significant distance in intercellular space before reaching its target cells.

2951-Pos Board B106
Size Distribution Analysis of Von Willebrand Factor
Svenja Lippok[1], Tobias Obser[2], Valentin Stierle[1], Ulrich Budde[3], Reinhard Schneppenheim[2], Joachim O. Rädler[1].
[1]LMU Munich, Munich, Germany, [2]Department of Pediatric Hematology and Oncology, University Medical Center, Hamburg-Eppendorf, Germany, [3]Medilys-Laboratory, Asklepios Clinic Altona, Hamburg, Germany.
Von Willebrand Factor (VWF) is a multimeric protein that promotes primary hemostasis, a process that highly depends on the size of VWF. Although in recent years a general understanding of VWF function has emerged, its dynamic size regulation remains rather unexplored. To this end, we investigate the size distribution of recombinant eGFP-VWF using Fluorescence Correlation Spectroscopy (FCS), Total Internal Reflection Fluorescence Microscopy (TIRF) and Quantitative Gel Analysis and find consistently an exponential decay in polymer size for plasma VWF. By monitoring the size distribution of VWF, we measure the *in vivo* and *in vitro* size regulation of VWF by the protease ADAMTS13, the major factor determining VWF function. We show that FCS is well suitable to quantify the ADAMTS13 induced cleavage rates and enable to discriminate between physiological and pathological concentrations of the protease in patient samples. In addition, we aim to develop shear-flow dependent measurements of the VWF cleavage. A two-focus FCS combined with a microfluidic device will serve as a fast flow-through setup to investigate shear-induced changes of the VWF functionality.

2952-Pos Board B107
Quantitative Characterization of Reversible GKAP-DLC2 Interactions in Live Neurons by Scanning Number and Brightness
Enora Moutin[1], Caroline Clerté[2], Vincent Compan[1], Fabrice Raynaud[1], Matthew L. Ferguson[2], Laurent Fagni[1], **Catherine A. Royer**[2], Julie Perroy[1].
[1]IGF/INSERM/CNRS/UM1/UM2, Montpellier, France, [2]CBS/INSERM/CNRS/UM1/UM2, Montpellier, France.
GKAP is a core protein of the scaffolding complex linked to glutamate receptors at excitatory synaptic synapses that enables their adequate targeting and function. GKAP interacts with DLC2, a light chain of molecular motors. In this work we performed a quantitative characterization of DLC2-GKAP interaction in living neurons using two photon scanning number and brightness (N&B) to decipher the molecular mechanisms sustaining glutamate receptor function in synaptic transmission. Cross-brightness measurements of mcherry-DLC2 and CFP-GKAP in living neurons highlight different degrees of homo- and heteromeric oligomerization depending on sub-cellular neuronal compartments. In dendritic spines, DLC2-GKAP interaction involves hetero-oligomers mainly composed by 2 DLC2 and 2 GKAP monomers, and some higher order species. Intracellular pools contained higher order homo-oligomeric complexes of GKAP and DLC2, with lower cross brightness, while